CellPress
Cancer Cell
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Compression of Pancreatic Tumor Blood Vessels by Hyaluronan Is Caused by Solid Stress and Not Interstitial Fluid Pressure

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http://dx.doi.org/10.1016/j.ccr.2014.06.003

Impaired perfusion is a hallmark of solid tumors that promotes progression, immunosuppression, and treatment resistance and is partly caused by vascular compression from excessive extravascular stresses (Chauhan et al., 2013; Stylianopoulos et al., 2012). The extravascular stress exerted by fluid is referred to as interstitial fluid pressure (IFP) and that by solid components as solid stress (SS). IFP is near zero in most tissues, but rises in tumors as impaired lymphatics fail to drain fluid leaking from blood vessels and IFP equilibrates with microvascular pressure (MVP) (Boucher and Jain, 1992). Due to equilibration, IFP in tumors can only transiently exceed MVP and thus cannot compress tumor vessels (Figure S1A available online). SS is generated as cells push and pull on their surroundings during proliferation and migration and is transmitted by extracellular matrix (Stylianopoulos et al., 2012). SS is greatly and chronically elevated in tumors due to high cell and matrix densities and can compress blood vessels (Figure S1A). Since the causes and consequences of elevated IFP and SS are different, strategies for alleviating these mechanical stresses are likely to be distinct.

In their article, Provenzano et al. elegantly showed that hyaluronan (HA) can mechanically compress blood vessels in pancreatic ductal adenocarcinoma (PDA) (Provenzano et al., 2012). However, their proposed mechanism—that HA leads to very high IFP that collapses vessels—is not consistent with the physiology of fluid homeostasis and calls for careful assessment of IFP in PDAs. They suggest that mean IFP can reach 99 mmHg (range 75–130 mmHg), presumably higher than MVP, in the *Pdx1-Cre/Kras$^{LSL\text{-}G12D/+}$/p53$^{LSL\text{-}R172H/+}$* (KPC) PDA model based on measurements made with a piezoelectric probe. To evaluate this, we measured IFP in KPC tumors with the wick-in-needle technique—which has been validated against the gold-standard micropipette technique (Boucher and Jain, 1992). We further measured IFP in additional PDA models, *Ptf1-Cre/Kras$^{LSL\text{-}G12D/+}$/p53$^{L/+}$* (KPdC) and *Ptf1-Cre/ROSA26-LSL-rtTA-IRES-GFP/Kras$^{TetO\text{-}LSL\text{-}G12D/+}$/p53$^{L/+}$* (iKPdC), which highly express HA (Figure S1B). The mean IFP was 8.1 mmHg (range 4.7–10.9 mmHg) in KPC, 3.4 mmHg (range 1.6–5.6 mmHg) in KPdC, and 6.7 mmHg (range 6.1–8.0 mmHg) in iKPdC (Figure S1C)—over an order of magnitude lower than the IFP levels reported by Provenzano et al. We also measured IFP with wick-in-needle in the tumors of four treatment-naive PDA patients (Figure S1C), and the mean IFP was 11.8 mmHg (range 6.1–16.6 mmHg). As these IFPs do not exceed typical MVPs, IFP cannot compress PDA vessels, leaving SS as the primary cause.

Provenzano et al. also propose that PDA IFP is not driven by equilibration with MVP because PDA vessels are nonleaky, i.e., nonpermeable to macromolecules. As evidence, they state that PDA IFP measured with the piezeoelectric probe remains elevated upon cardiac cessation, indicating that blood pressure is not driving IFP. With wick-in-needle, we found that cardiac cessation reduced IFP to zero in all three PDA models, confirming that blood pressure drives elevated IFP (Boucher and Jain, 1992). Furthermore, their hypothesis that PDA vessels are nonpermeable to macromolecules is contradicted by their own data—PEGylated recombinant human hyaluronidase (PEGPH20), a macromolecule, clearly permeates across PDA vessels since it acts on interstitial HA. Moreover, the efficacy in PDA patients of nanoparticle-albumin-bound-paclitaxel, an FDA-approved macromolecule, also indicates that PDA vessels are somewhat leaky. We conclude that PDA IFP is indeed driven by blood pressure and that fluid exchange between the intravascular and interstitial space in PDA facilitates equilibration of IFP and MVP as in other tumors.

The discrepancy between our IFP measurements and those of Provenzano et al. may stem from their use of the piezoelectric probe technique (Ozerdem and Hargens, 2005), which we believe suffers from artifacts coming from SS. As evidence for our hypothesis, Provenzano et al. measured an IFP of 10.4 mmHg (range 8–13 mmHg) in normal mouse pancreata, although normal murine tissues typically have slightly negative IFPs. Ozerdem and Hargens tested this technique against wick-in-needle in a single tumor model in two mice, but they did not carefully test for such artifacts—for example by comparison to wick-in-needle in tissues

of varying matrix or cell density. We therefore compared the piezoelectric probe technique to wick-in-needle in multiple normal murine tissues. We found that the piezoelectric probe produces significantly higher measurements when compared with wick-in-needle (Figures S1D and S1E). For example, we measured pancreas IFP as −0.7 mmHg (range −0.9 to −0.5 mmHg) with wick-in-needle, whereas our pancreas measurements with the piezoelectric probe were 9.8 mmHg (range 8.5–11.3 mmHg). This discrepancy most likely occurs because the sensor in the piezoelectric probe, unlike that in wick-in-needle, directly contacts cells and matrix allowing solid tissue components to contribute to the reading. We conclude that the piezoelectric probe method developed by Ozerdem and Hargens—and used by Provenzano et al.—measures pressures that are higher than the actual IFP due to artifacts from solid tissue components.

As demonstrated here, HA-rich desmoplasia in PDA does not produce unusually high IFP, IFP cannot compress PDA vessels, and the technique Provenzano et al. used to measure IFP actually measures a combination of IFP and SS. Meanwhile, we found that HA increases SS through storage and transmission mechanisms (Stylianopoulos et al., 2012). Thus, PEGPH20 most likely reduces SS, thereby decompressing vessels. Since IFP cannot compress blood vessels, we propose that the mechanism of vessel decompression by PEGPH20 is solely a reduction in SS. Importantly, therapies that alleviate SS cause decompression of vessels and improve PDA treatment (Chauhan et al., 2013). Thus, we concur that PEGPH20 has immense promise for PDA, and we hope that this correspondence clarifies its mechanism.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and one figure and can be found with this article online at http://dx.doi.org/10.1016/j.ccr.2014.06.003.

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